NONSPECIFIC AND COOPERATIVE BINDING OF LECTINS TO MICROORGANISMS

LEO D. KAHN

Eastern Regional Research Center, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118

• *Binding of lectins to microbial cell walls was investigated by fluorimetric titration and Scatchard plot. Data were correlated with agglutinability. Concanavalin A and lectins of wheat germ, soybean, pea, lentil, and peanut were tested against* Escherichia coli, Micrococcus luteus, Lactobacillus plantarum, *and* Bacillus subtilis. *In cases where binding occurred, it was either nonspecific or positively cooperative. Agglutination was observed only in those combinations of lectin and microorganism that showed positive cooperative binding, suggesting a definite relation between binding and agglutination. Lectins binding to the same carbohydrate did not necessarily bind to the same microorganism, confirming the complexity of the lectin-receptor bond.*

INTRODUCTION

A degree of specificity of lectins in binding to biological cells and in inducing agglutination suggests that these proteins may be useful in the detection and identification of microorganisms. Development of methodology with lectins used to selectively isolate and concentrate microorganisms should make it possible to supplement classical microbiology with physicochemical techniques and in some cases even eliminate the need for time-consuming culturing of microorganisms. Moreover, investigation of the binding sites and the nature of the binding forces should lead to further understanding of microbial cell wall structure, the antigen-antibody reaction, and possibly to new concepts of gram positive/gram negative distinction among microorganisms. It was with these ends in view that the present study of the relation between the binding of lectins to microorganisms and agglutination was carried out.

MATERIALS AND METHODS

Four prokaryotic microorganisms were titrated fluorimetrically vs. lectins labeled with fluorescein isothiocyanate. The microorganisms were suspended in 0.01 M tris buffer made 0.15 M in NaCl and 0.001 M in CaCl, $MgCl_2$, and $MnCl_2$. The stoichiometric data obtained were arranged in Scatchard plots and correlated with agglutinability. The validity of the use of fluorescence in this type of measurement was established by Monsigny et al.,[1] who showed that data thus obtained agreed with the data obtained by radioassay.

The original microorganism cultures, supplied by the Northern Regional Research Center of the U.S. Department of Agriculture, were *Lactobacillus plantarum* (B-5461), *Escherichia coli* (B-3704), *Bacillus subtilis* (NRS-744), and *Micrococcus luteus* (B-1018). The *E. coli* and the *B. subtilis* were grown for 24 h at 30°C; and the *M. luteus* for 24 h at 25°C. In this assortment both gram positive and gram negative organisms are present as well as both flagellate and nonflagellate species. The microorganisms were grown in conventional media, washed free of all extraneous material, and finally lyophilized.

The tagged lectins used were concanavalin

A (Con A), soybean agglutinin (SBA), wheat germ agglutinin (WGA), lentil agglutinin (LA), pea agglutinin (PA), and peanut agglutinin (PNA), all purchased from Pierce Chemical Company, Rockford, Illinois.*

The assay was carried out on 80 mg masses of microorganisms to which were added a series of aliquots of tagged lectin ranging from 2.5 μg to 36.0 μg with the final volume made up to 5.0 ml with the buffer. After an incubation period the lectin-microorganism complex was separated by centrifugation, and the unbound lectin in the supernatant liquid was determined fluorimetrically. A low molecular weight carbohydrate specific for the lectin was added to the lectin-microorganism complex and, after incubation and centrifugation, the recovered lectin was measured in the fluorimeter. A material balance for the lectin was then drawn up.

Agglutination was detected by microscopic examination of a hanging drop of the lectin-microorganism complex. All processes were carried out at room temperature.

To insure that the binding was reversible, portions of the lectin-microorganism complexes were placed in contact with the buffer; in all cases the lectin was completely recovered.

*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over other suppliers not mentioned.

RESULTS

The stoichiometric data were arranged in Scatchard plots,[2] two of which are shown in Figs. 1 and 2. In a Scatchard plot the ratio of bound to total sites relative to the concentration of unbound ligand at equilibrium is plotted vs. the ratio of bound to total sites. In the figures, r is the ratio of mass of bound lectin to mass of microorganism present, and A is the mass of unbound lectin. Since all samples were made up to the same volume, the ordinates and abscissae plotted here are proportional to the ones used in a conventional Scatchard plot and therefore yield a curve of the proper shape. Figure 1 gives the Scatchard plot for the binding of WGA to *L. plantarum*. The curvature and positive slope

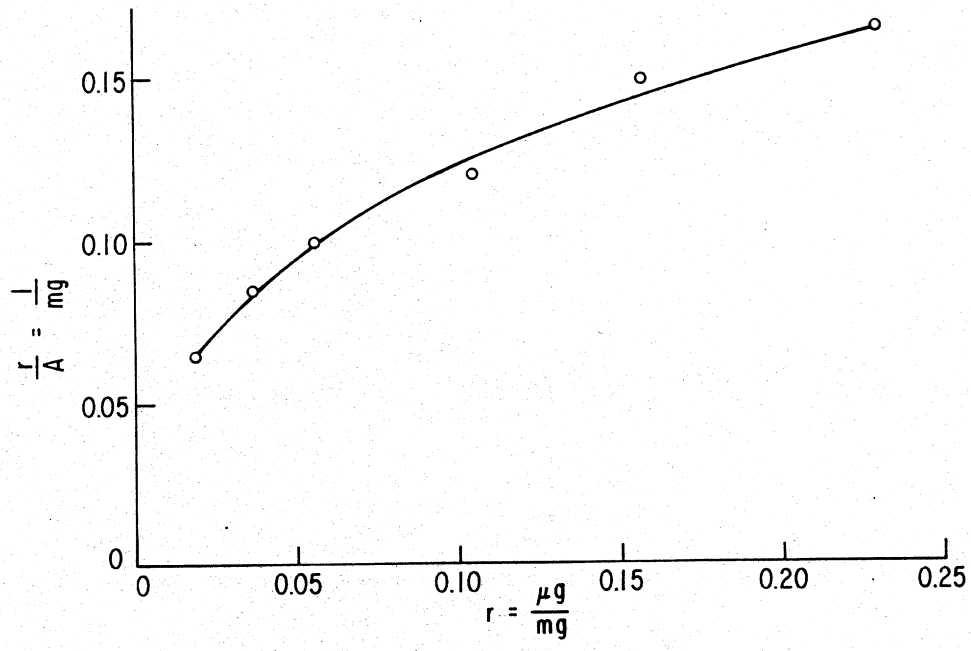

FIGURE 1. Scatchard plot for wheat germ agglutinin bound to *Lactobacillus plantarum*.

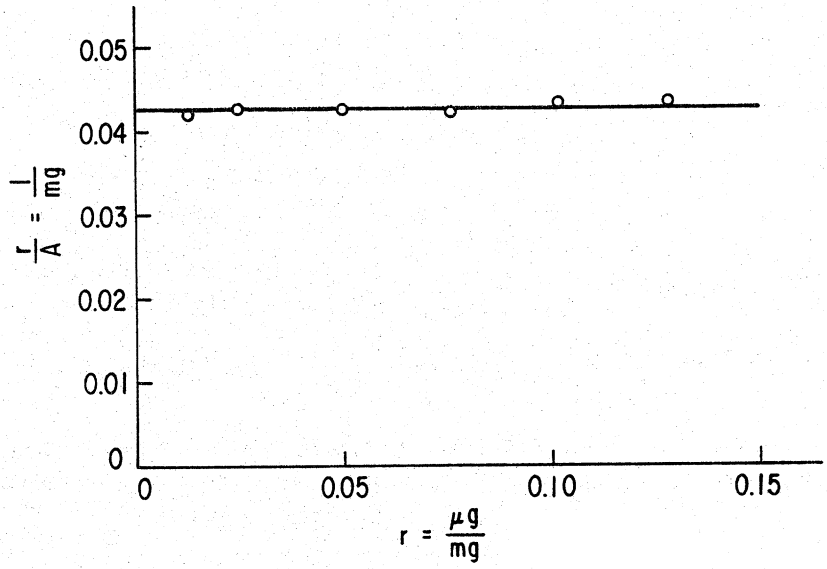

FIGURE 2. Scatchard plot for soybean agglutinin bound to *Micrococcus luteus*.

TABLE I. Binding and Agglutination of Lectins to Microorganisms[a,b]

Lectin	*L. plantarum*	*E. coli*	*B. subtilis*	*M. luteus*	Specific carbohydrate
Con A	(−) Nonspecific	(±) No binding	(++) Pos. coop.	(−) Nonspecific	Me-α-D-mannopyranoside
SBA	(−) Nonspecific	(±) No binding	(−) No binding	(−) Nonspecific	N-Ac-D-galactosamine
PNA	(±) No binding	(±) No binding	(−) No binding	(±) No binding	N-Ac-D-galactosamine
WGA	(++) Pos. coop.	(++) Pos. coop.	(+) Pos. coop.	(+) Pos. coop.	N-Ac-D-Glucosamine
LA	(−) No binding	(−) No binding	(−) No binding	(+) Pos. coop.	Glucose
PA	(−) No binding	(−) No binding	(−) No binding	(−) No binding	Glucose

[a]Types of binding: nonspecific, no binding, or positively cooperative. [b]Extent of agglutination: (++) nearly all cells agglutinated; (+) definite agglutination; (±) practically no agglutination; (−) definitely no agglutination.

show this to be a case of positively cooperative binding.[3] Figure 2 is the Scatchard plot for the binding of SBA to *M. luteus*. The straight line of zero slope indicates that this is a case of nonspecific binding.[4]

Results are summarized in Table I, which shows the type of binding and extent of agglutination of each combination of lectin and microorganism. It also shows the low molecular weight carbohydrate used as a

competitor. The competitor removed lectin from the lectin-microorganism complex even in cases of nonspecific binding, and no binding occurred in the presence of competitor.

From Table I it is seen that in the systems studied the lectins bound selectively to the microorganisms, with only positively cooperative and nonspecific binding in evidence. Agglutination occurred only when the binding of lectin to microorganism was positively cooperative.

Lectins that bind to the same carbohydrate do not necessarily bind to the same microorganism. This is seen in the cases of LA and PA. Both these lectins bind to glucose but only LA binds to *M. luteus.* Similar behavior is seen for SBA and PNA, where both bind to N-AC-D-galactosamine, but only SBA binds to *M. luteus* albeit this is a case of nonspecific binding.

Removal of lectin from the lectin-microorganism complex by competitor had various effects. In the case of WGA bound to *L. plantarum,* definite but not complete disaggregation took place. In the cases of WGA bound to *B. subtilis* and to *E. coli,* the agglutination persisted. The aggregates of the Con A-*B. subtilis* complex disaggregated, but subsequently the microorganisms formed chained structures. With WGA or with LA bound to *M. luteus,* there was limited disaggregation.

From these observations it can be concluded that the binding of a lectin to a microorganism is not a simple process. It is certainly not a simple case of covalent binding to a carbohydrate. Evidently some combination of bonds occurs, and one particular lectin may enter into different kinds of binding with different substrates.

DISCUSSION

In agreement with the data presented here, Lotan et al.[5] reported agglutination of *E. coli* and *M. luteus* cells by WGA,[5] and Birdsell et al.[6] reported agglutination of *B. subtilus* cells by Con A. Picken and Beacham observed that Con A agglutinates *E. coli* mutants but not the parent cells[7] and SBA does not agglutinate *E. coli.* On the other hand, contrary results have been demonstrated by Hamada et al.,[8] who reported agglutination of *L. plantarum* cells by Con A; and Archibald and Cooper,[9] who achieved agglutination of *L. plantarum* cell walls but not of *B. subtilus* cell walls with Con A. Also, Picken and Beacham[7] did not observe agglutination of *E. coli* by WGA. It is possible, however, that these discrepancies are due to strain specificity of the lectins.

No claim is made that positively cooperative binding of lectins to microorganisms invariably leads to agglutination, because this effect has been established only for the systems herein reported. Maruyama[10] has stated that the binding of lectins to cells and agglutination are independent events, since in his experiments protease-treated *E. coli* spheroplasts agglutinated in the presence of Con A whereas untreated spheroplasts did not, even though both bound the same amount of lectin. In this instance the nature of the binding was not investigated, hence it is possible that it was nonspecific.

The data presented here indicate that at this stage of the research a pattern of promising purport appears to be emerging. Whether that pattern is universal is a question that will be investigated in this laboratory by other techniques. □

REFERENCES

1. M. Monsigny, C. Sene, and A. Obrenovitch. Quantitative fluorimetric determination of cell-surface glycoconjugates with fluorescein-substituted lectins. *Eur. J. Biochem., 96,* 295 (1979).
2. G. Scatchard. The attraction of proteins for small molecules and ions. *Ann. NY Acad. Sci., 51,* 660 (1949).
3. W. F. Dahlquist. The meaning of Scatchard and Hill plots. *Methods Enzymol., 48,* 270 (1978).

4. E. Winkler and G. Huebner. Sources of error in the analysis of complex binding systems by non-linear Scatchard plots. *Stud. Biophys., 66,* 211 (1977).
5. R. Lotan, N. Sharon, and N. Mirelman. Interaction of wheat germ agglutinin with bacterial cells and cell-wall polymers. *Eur. J. Biochem., 55,* 257 (1975).
6. D. C. Birdsell, R. J. Doyle, and M. Morgenstern. Organization of the teichoic acid in the cell wall of *Bacillus subtilis. J. Bacteriol, 121,* 726 (1975).
7. R. N. Picken and I. R. Beacham. Bacteriophage-resistant mutants of *Escherichia coli* k 12 with altered lipopolysaccharide: studies with concanavalin A. *J. Gen. Microbiol., 102,* 319 (1977).
8. S. Hamada, K. Gill, and H. D. Slade. Binding of lectins to *Streptococcus mutans* cells and type-specific polysaccharides. *Infect. Immun., 18,* 708 (1977).
9. A. R. Archibald and H. E. Coapes. The interaction of concanavalin A with teichoic acids and bacterial walls. *Biochem. J., 123,* 665 (1971).
10. H. B. Maruyama. Agglutination of bacterial spheroplast. *J. Biochem. (Tokyo), 75,* 165 (1974).

(Received December 12, 1981)